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Introduction and Rationale

Coral formations that make up the Florida Keys barrier reef are near the northern limits for Atlantic coral reefs and are subject to greater natural environmental stress than corals on reefs in more southern locales. In addition, the Florida Keys barrier reef is impacted directly by extensive human visitation, fishery uses, and by proximity to great human population densities.

Disease epidemics have affected the reefs also, including coral diseases and the epidemic dieoff of the long-spine sea urchin, Diadema antillarum. These urchins previously consumed reef algae, and in the absence of the urchins there is more algae on the reefs to compete with corals. This is one factor among many that has caused the decline of corals on the Key's reef tract. There is a body of scientific literature that supports the idea that the long spined sea urchins acted as a keystone species on the reefs of the Caribbean and Florida Keys. Prior to 1983, these urchins occurred in vast numbers (1 to 4 per m² or greater) on almost every reef from near shore patches to the deep reefs. A plague of viral or bacterial origin, swept through the Caribbean, Bahamian and Florida reefs in 1983-84. Within a matter of months almost every *Diadema* on the Florida Reef Tract and throughout the Bahamas and the Caribbean had succumbed and the reefs lost a most important element of algae growth control.

There are now some small natural populations of *Diadema* on the reefs throughout the Keys. A few adults can be found scattered about various reefs areas, but population densities are very low (<0.01 individuals per m²) and there are few adults (>5 cm test diameter). Remaining adults may be resistant to the plague organism or the plague organism may no longer be present in these waters - nobody knows. Importantly, some natural recruitment of *Diadema* occurs in June through September of each year. Many

hundreds of small juveniles can be observed in shallow water rubble zones around Conch Key in late summer and early fall (Ken Nedimyer, personal observation). The survival of these small *Diadema* may be due to an abundance of the proper habitat of small, loose rocks with open crevices of the right size for juvenile protection and lack of predators in these shallow areas. Predation of small, post larval *Diadema* on deeper reefs may be extremely severe due to the absence of large urchins, but no recruitment data exist for deeper reef areas. It is also possible that large urchins may create protected "predation free" sites within their habitat where post larval urchins can survive until they are large enough to repel predators on their own, but this remains a hypothesis.

One thing is certain, the grazing activity of large urchins cleans the substrate of algal growth and creates a favorable habitat for settlement of corals. Lessios (1988) also speculates that the grazing activity of the adults, which cleans the substrate of most algae growth, may make post larval Diadema settlement more likely to occur on reefs with larger adult populations. The juveniles that settle in the rubble zones around the shallow, offshore reefs appear not to survive though fall and winter, which, in our opinion, is due to the impact of storm events on this shallow rubble habitat. We hypothesize that these juveniles survive and grow for several months on shallow rubble areas until the storm events of fall and winter, during which the wash and movement of the rubble destroy these small urchins during the storms. Although limited, it appears that some additional annual recruitment of juveniles also occurs on low profile hard bottoms in deeper areas.

One factor limiting the natural return of *Diadema* to these reefs may be low adult population density. Experimentation with Pacific sea urchins has shown that when more than a meter separates adults, fertilization of eggs during spawning is very low. Lessios (1988) refers to the work of the Pennington (1985) who reports that the distance at which sperm can successfully fertilize eggs in echinoids is only 20 cm from the spawning male. Lessios (1988) then reasonably assumes that relatively high densities of adult Diadema may be necessary for optimal natural reproduction. These high population densities, once common to Florida reefs, are no longer present. Natural repopulation of *Diadema* on these reefs may occur over time, but this repopulation time may be measured in decades if not centuries. Even though the loss of *Diadema* sea urchins on the reefs of Florida Keys was a natural event affecting a vast area, it may be possible, and certainly beneficial to the condition of the coral communities on our reefs, to expedite the return of *Diadema* to the reefs. Even though adult numbers are small, new recruits still appear predictably in certain habitats and at certain times of the year, as described above.

We propose to artificially enhance population densities of *Diadema* through transport of large numbers of small juvenile urchins to specific reef locations. If successful, additional projects might include spawning and growing urchins to early juveniles under hatchery conditions and then releasing them on specific reef locations (hatchery work is already underway by Dr. Alina Szmant, with a SeaGrant project). Such projects would serve to increase population densities of urchins in areas where they might be most needed and

produce increased densities to enhance spawning success in future years. Repopulation of the Florida reef tract with these efficient herbivores should reduce algae competition with coral and improve the growth and settlement of many species of coral and other species of sessile invertebrates.

This project will be conducted in several phases. This will keep effort and financial expenditures within reasonable limits and allow analysis of initial results to guide development of future phases of the project.

It may be that replacement of this keystone species on the reefs of the Florida Keys is the single most beneficial project that can be done to improve conditions on these reefs. First, it must be shown that "normal" population densities of *Diadema* sea urchins on reef structures will improve coral growth and reef conditions, and that transplanted urchins will survive and grow on patch reef structures. This initial phase of the project can be done simply and inexpensively. The goal of this proposal is to determine if transplanted juvenile urchins survive, and what effect these urchins have upon a patch reef that previously had few, if any, *Diadema* in residence (absence due to historical dieoff and not habitat unsuitability).

References:

Lessios, H. A. 1988. Mass Mortality of Diadema antillarum in the Caribbean: What have we learned? Ann. Rev. Ecol. Syst. 19: 371-393.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation and synchronous spawning. Biol. Bull. 169: 417-430.

Hypotheses

 H_1 : It is possible to transplant large numbers of small *Diadema antillarum* from shallow rubble zones to deeper patch reefs.

H₂: Increasing the densities of *Diadema antillarum* to approximate pre-plague levels on small, isolated patch reefs will facilitate a reduction of algal overgrowth and will enhance coral growth and settlement.

Project Protocol Summary

Four similar small patch reefs at the same depth in the same general area in the upper Florida Keys will be selected for study. Transfer *Diadema* sea urchin recruits from scattered natural populations found in rubble areas to two of these patch reefs. Assess

and document the condition of the experimental and reference (control) reefs (algae, corals, sponges, etc.) before urchins are translocation, and for a period of at least one year after translocation.

Research Goals

- 1. Determine if *Diadema* urchins survive transplantation and the size that exhibits the best survival rate after transplantation.
- 2. Estimate the survival rates and the growth rates of transplanted *Diadema*.
- 3. Determine the distribution patterns that *Diadema* urchins develop on the experimental reef. (They will be placed initially in protected microhabitats within the reef structure and this initial distribution will be recorded on maps of the patch reefs.)
- 4. Compare and contrast general reef condition and community level changes, including coral recruitment and growth, on the experimental and control reefs over time.

The National Undersea Research Center (NURC/UNCW) at the University of North Carolina will conduct their Rapid Assessment protocols to the reference (control) and experimental reefs on two occasions, once just before translocation of the urchins and once about one year after translocation. If results warrant, an additional assessment will be made at three and six months. NURC/UNCW will also be conducting surveys of dozens of other patch reefs in the Florida Keys during 2001 to provide context to this experiment. The parameters to be collected will include:

The Rapid Assessment Parameters (NURC/UNCW):

- 1. Percent cover (abiotic and biotic components) of benthos
- 2. Topographic complexity
- 3. Urchin density and size by species (based on transects but other surveys are in more detail and include maps)
- 4. Gorgonian density and height
- 5. Species richness of corals, gorgonians and sponges
- 6. Stony coral density, size and condition
- 7. Juvenile coral density and size
- 8. Spiny lobster (and other urchin predators such as *Mithrax*) density
- 9. Video archive (Video photo documentation used only for archive purposes.)

Methods and Procedures

A log will be kept of each work visit to the patch reef sites. The number of workers, number and time of dives, and work accomplished at each visit will be logged for reference of effort expended.

- 1. Find and delimit four small patch reefs inshore of the Conch Reef area that are similar in size, general location, and depth and designate two as experimental and two as control. Identify and delimit these locations with GPS readings. Two control and two experimental reefs, each small, about the same size, and isolated by surrounding grass beds and sand flats, will provide the replication necessary for statistical analysis of the data. (Every effort will be made to maintain two reference (control) and two experimental reefs in the study. One experimental reef will be used only if both experimental reefs cannot be supplied with more than 10 urchins per square meter.)
- 2. Survey and map each of these small reef areas locating any existing *Diadema* urchins and recording the location of existing large coral and rock formations. Determine the size in square meters of each of these small reef areas. These simple maps will be transcribed on plastic work slates and used to mark numbers and locations of *Diadema* on future visits.
- 3. Establish three sites of 1 m^2 on experimental and control patch reefs that will be used as permanent photo-quadrats for photography of reef conditions over the term of the project. Photographs will be useful for press releases and presentations, should the transplants work.
- 4. Obtain independent analysis of the pre-experimental condition of each selected reef site (conducted by NURC/UNCW, see above).
- 5. Determine, if possible, pre-plague densities of *Diadema* on the Florida reefs.
- 6. Determine the locations and density of existing natural populations of *Diadema* at reef and rubble locations where significant settlement of this species has naturally occurred.
- 7. Develop safe and effective methods of transport of *Diadema* to other locations. (Ken Nedimyer has experience with collection and transport of *Diadema* urchins.)
- 8. Develop methods for estimation of size of *Diadema* (3-4 size classes will be used so we don't have to handle urchins to obtain measurements) useful in analysis of transplant success, survival, and growth. Size differences of a magnitude that indicate different year classes will be especially noted.

9. Once the project has permits from the Sanctuary, and the initial preparations are completed, the principal and associate investigator will move as many *Diadema* urchins as is necessary to the experimental reefs and record the numbers and sizes of urchins moved. The actual number of *Diadema* transported to the experimental reef will depend on the area of the reef, and availability of urchins at the time of transplant. It is anticipated that the number of urchins placed on each experimental reef will be between 10 and 25 individuals per square meter. The maximum number of transplants will be attempted since mortality of the juvenile urchins is likely to be high. They will be placed in many locations on the experimental reefs in areas that appear favorable to survival. Depending on time of year and recent storm events, under the most favorable conditions it is estimated that movement of sufficient numbers of *Diadema* can be accomplished in less than one week. Depending on environmental conditions at the time that the project is permitted, it may be necessary to delay the transfer of *Diadema* to the experimental reef until conditions are favorable.

10. Over the course of the experiment, the control and experimental reef will be visited at intervals of about one to two months, as weather and work time make possible. In an effort to determine if predation has immediately reduced the numbers of transplanted small *Diadema*, the first visit to the project sites will be planned for a few days to a week after the transplantation is complete. This early count will provide an estimate of the initial survival of the transplanted urchins. The next survey will be made about three weeks after translocation and then, as possible, at monthly intervals over the following year. The size range of observed *Diadema* urchin on each reef area will be recorded on the map slate, and each of the established photo-quadrats will be photographed. The principal investigator will determine the most effective pattern of search on each patch reef once the reef areas are selected and mapped. The patch reefs will be well mapped and the path of the survey swims will be recorded on the plastic slate map to avoid any overlap of the survey.

Additional data such as time and date of each visit, general reef and water conditions, and general observations (including comments on fish and lobster populations and impressions of general reef conditions) will be recorded for each visit. A "roving diver technique" may be used to obtain an estimate of the species and abundance of fish populations on each site before transfer of *Diadema* to the experimental patch reefs and a few times after transfer, as conditions and time allow.

11. Once the experimental reef is seeded with the estimated proper number of juvenile and a few adult urchins, no further transport of *Diadema* urchins are planned. However, if small urchins do not survive the initial translocation, an additional translocation may be attempted with larger urchins if conditions permit. Monthly visits (in periods of bad weather or other contingencies the time between visits may be more extended than monthly) will be continued for at least a period of one year. NURC/UNCW will conduct an initial, pre translocation assessment of the condition of the four reefs (two reference

and two experimental) and a second assessment after a period of one year, perhaps sooner if urchin survival is high and grazing is apparent.

12. The associate investigator, with the collaboration of the principal investigator, will prepare two brief interim reports, the first two or three weeks after the initial seeding of *Diadema* on the experimental reef, and another about 6 months into the project. The comprehensive final report on the results will be completed after about one year of observations.

Proposed Project Duration

The time frame of the project is expected to run for 12 to 18 months. After initial preparations, the start of the project will depend on the presence of sufficient *Diadema* in the rubble zones and on the weather. It is anticipated that the translocation of small *Diadema* will occur in late summer of 2001. The determining factors are the presence and size of young urchins, and weather conditions. The principal investigator (Nedimyer) will monitor recruitment and size of *Diadema* during late spring and summer and determine when successful translocation may be conducted.

Project Time Line

Month 1 - 2 - Proposal preparation

Month 3 - Selection of experimental and control patch reef areas. Initial survey of *Diadema* populations in the Conch Reef area. (Estimated for April 2001)

Month 4 - 5 - Development of methods and demarcation of sites for photographic documentation of reef conditions on experimental and control reef area. Recording of baseline documentation of areas, GPS locations, mapping of reef areas, selection of sites for photographic documentation and taking of first baseline photographs of reef and coral condition, and recording of observations on fish populations. (Estimated at May and June 2001)

Month 6 - Transport of *Diadema*, juveniles and adults, to the experimental reef and a post transplant visit to the sites to estimate initial survival of the transplanted urchins. First assessment of reef condition by NURC/UNCW before translocation of urchins. First post translocation assessment of reefs one week after translocation. (Estimated at August 2001)

Month 7 - First monthly Diadema count, photographic documentation, and fish population observations. (Estimated at September, October 2001)

Month 8 - Second monthly *Diadema* count, photographic documentation, and fish population observations. (Estimated at November 2001)

Month 9 - 18 - Diadema counts, photographic documentation of reef and coral condition and fish population observations will continue monthly or as conditions allow. An interim report will be prepared about 6 months after initial transport of the urchins to the experimental sites and the final report will be prepared 12 to 14 months after the initial transport of the urchins. Post translocation assessment visit by NURC/UNCW.

Expected Results and Benefits

This project will provide insight into the growth, survival, and distribution of juvenile *Diadema antillarum* on reef areas through *in situ* observations and population inventories during the duration of the project. In the long term, we anticipate that reestablishment of "normal" densities of *Diadema* on an offshore patch reef will also result in better coral settlement and better reef condition. This project may also provide insight, through the monthly population inventories, into the extent of predation and degree of loss of juveniles on reef areas. Although the effects of a more natural population of *Diadema* on a patch reef should be evident after the first year, the beneficial effects of this population will increase in later years as the juveniles become adults and the effects of their algae grazing becomes greater. *Diadema* can become reproductive in a period of one year although full adult size has not yet been attained. Although success is difficult to predict, survival of 1 to 5 early adults per meter after a period of one year should result in greatly enhanced breeding success, enhanced settlement of post larval urchins, and a more favorable environment for coral growth and recruitment.

This project may identify a workable method to improve biodiversity on the reefs and enhance the health of the Florida reef tract by reducing algal growth in and around the reefs.

Proposed Budget

Personnel

The budget for this project includes dive time for the principal investigator, perhaps one week a month during the initial period of project development, thence one or two days a month during the routine conduct of the project. The associate investigator may also require some dive time during the initial period of project development and a day or two before each interim report. The interim reports will require a day or two of analysis and report preparation and the final report may require a week of preparation.

Personal costs are estimated at about \$300.00 per day. A rough estimate of the work days for the project is:

Principal investigator - 32 (including 30 dive days) Associate investigator - 15 (including 7 dive days)

Equipment and supplies

Basic equipment required for the study.

- 1. Boat and associated operational equipment.
- 2. Dive gear
- 3. 35 mm camera with flash
- 5. Video camera with underwater case
- 6. Tape measure and/or line for measuring reef areas
- 7. Hammer and SS spikes
- 8. PVC grid for use with photo documentation
- 9. 35 mm slide film (one or two rolls per month, perhaps a total of 30 rolls of 36 exposure Kodachrome film)

The principal investigator already owns most of this equipment, including a suitable boat and associated gear. Purchase of equipment will not be charged to the project. A reasonable rental fee for this equipment, plus equipment operational expenses (gas, oil, etc.) to the project is estimated at \$200.00 per day. It is estimated that the number of dive days for the project will be about 30.

The associate investigator owns the computer equipment necessary for data recording and basic analysis related to urchin densities and sizes, and for generation of the interim and final reports. The costs associated with computer equipment and time are included in the personal charge for the associate investigator's time.

The estimation for the total costs of the project, time and equipment rental is:

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Personnel time - 47 days at $300.00 per day — $14,100.00
Equipment rental (dive days) - 30 days at $200.00 per day — $6,000.00
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Independent scientific analysis of reef conditions

Meetings have been held with NURC/UNCW and they have a strong interest in this project. They have agreed to perform the Rapid Assessment Parameters study for this project, including data analysis and report writing for \$6,000.00.

Estimate of total project expenses — \$26,100.00

Travel expenses

Travel expenses are not anticipated at this point. It may be necessary, however, for the principal and/or associate investigator to travel to a distant location for presentation of results or library research. Some of the personnel time listed above may be applied to limited travel if necessary and possible. Funding for any extended travel, however, will have to come from other sources.

Additional scientific services \$2,000.00

It may be necessary to employ additional outside help scientific consultations, such as for fish censuses, or lab analyses if the urchins start to die in the same way as during the disease epidemic.

Total project budget - \$28,100.00 graphical Data

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Resume attached

Associate Investigator

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